Molecular and Ontogenic Analysis of the Human Sperm Tail Fibrous Sheath

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ABSTRACT

In the past few years, a considerable amount of data has been obtained regarding the biochemical and antigenic structure of the human sperm tail fibrous sheath (FS). The development of a method for the FS isolation enabled its biochemical characterization, and several polypeptides with MW ranging between 25 and 97 kDa were identified. These were antigenic, and their dissection with monoclonal antibodies (MoAbs) showed the presence of bioactive groups e.g. phosphates and sugars. The bioactive groups were incorporated into the proteins late in spermatogenesis, following the FS assembly, as additional steps of post-translational modifications; this ensures structural maturity of the sperm tails prior to the sperm release to the epididymis. This chapter is a description of these results and their significance.

RÉSUMÉ

Analyse moléculaire et ontogénétique de la gaine fibreuse de la queue du spermatozoïde humain

Au cours des dernières années, une quantité considérable d'informations a été obtenue à propos de la structure biochimique et antigénique de la gaine fibreuse de la queue du spermatozoïde humain (GF). Le développement d'une méthode d'isolation de la gaine fibreuse a permis sa caractérisation biochimique, et plusieurs polypeptides, de poids moléculaire entre 25 et 97 kDa ont été identifiés. Ces polypeptides sont antigéniques, et leur analyse grâce à des anticorps monoclonaux a montré la présence de groupes biologiquement actifs tels que des phosphates et des sucres. Les groupes biologiquement actifs sont incorporés dans les protéines à un stade tardif de la spermatogenèse, après l'assemblage de la GF, grâce à des étapes additionnelles de modifications post-traductionnelles. Ceci garantit la maturité structurale de la queue du spermatozoïde avant la libération du sperme dans l'épididyme. Ce chapitre décrit ces résultats et leur signification.

The fibrous sheath (FS) is a unique cytoskeletal structure of the sperm tail which occupies the principal piece. Its two longitudinal columns run dorso-ventrally along the outer dense fibres numbers 3 and 8, and terminate by attaching to axonemal microtubular doublets numbers 3 and 8. These columns are interconnected by large numbers of transverse ribs [9]. During spermiogenesis, the FS assembly of the human spermatozoon begins at the fourth Sc stage spermatid by the formation of a network of filamentous tubules [8] which are later obliterated through the deposition of electron dense granular material.

Until recently, nothing was known of the molecular or antigenic structure of the human sperm tail FS despite its involvement in various flagellar abnormalities which are associated with sperm immotility and infertility [6, 18, 19, 21]. Molecular studies, therefore, are important to

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understand the biological significance of the FS in sperm motility and to unravel the molecular basis for its anomalous formation. Furthermore, an understanding of the protein synthesis and assembly could lead to the development of new methods of contraception through identifying reagents capable of disrupting these processes without affecting other tissues. Therefore, in the past few years, the main interest of the author was focused on this subject, and the results of these studies are described below.

RESULTS AND DISCUSSION

Biochemical and antigenic analysis of the FS

The development of a method for isolating highly purified human FS preparations enabled biochemical analysis of the FS [22]. As in rodents [5, 10, 26], the SDS-PAGE of human preparations revealed several major protein bands (Fig. 1) with MW ranging between 25 and 97 kDa [22]. The presence of several polypeptide bands is a reflection of the FS structural complexity, although some of these peptides could be degradation products. It is possible that different proteins are utilized in the construction of the various FS components such as the transverse ribs and longitudinal columns, which develop independently [13] and are made up of different sized tubules [9, 28], and the electron dense material deposited in these filaments. In this respect, the FS is similar to neurofilaments whose structure involves the use of three different but closely related polypeptides which are encoded by three genes [30]. However, amino acid sequencing of the FS proteins is essential to determine any sequence homology.

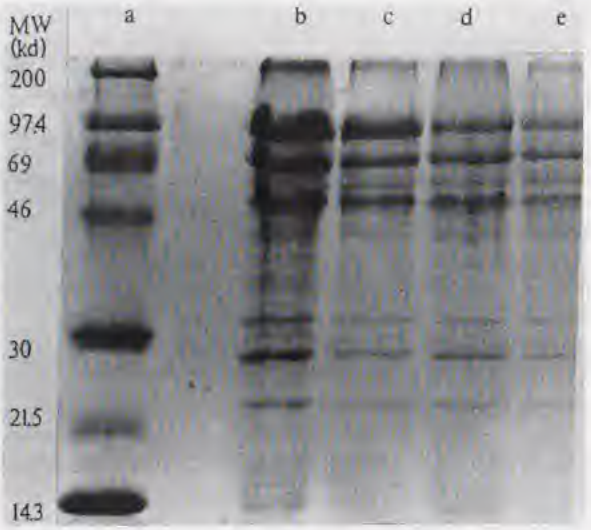

FIG 1. — SDS-PAGE of purified human sperm fibrous sheath (FS) obtained from four normozoospermic donors showing several protein bands, lanes (b-e); a, MW markers. From [22], reproduced by permission of *Human Reproduction*.

The FS polypeptides are highly immunogenic and express antigenic epitopes which are not shared by several somatic tissues. This was demonstrated by using mouse xenoantisera (MAFA) which were raised against purified human sperm FS and stained seven major protein bands (Fig. 2) with MW ranging between 25 kDa and 97.4 kDa by Western blotting [23]. In humans, MAFA did not react with other sperm tail cytoskeletal elements, but in rats, a cross-reaction between the FS and outer dense fibre proteins was reported [26].

In addition to xenoantisera, MoAbs have also been used to investigate the FS antigens. AJ-p97 was the first polypeptide to be identified using RT97 MoAb [14] which was later found to be phosphorylated [20]. In rats, the 80 kDa phosphoprotein reported by amino acid analysis [5] could be analogous to AJ-p97, although amino acid sequencing is required. Other MoAbs included GDA-J/F3 which detected a non-collagenous asialo-glycoprotein [16], AJ-FS1 and AJ-

FS9 MoAbs which recognized several FS-specific antigens [15, 17]. However, unlike other MoAbs, the AJ-FS9 detected masked antigens which required proteolytic enzymes treatment for their exposition; this indicates that the FS assembly involves the deposition of proteins mutilayers [15]. In Western blotting, the reaction of AJ-FS1 and AJ-FS9 with multiple protein bands did suggest the presence of shared epitopes in these polypeptides and possibly some structural homology. Another group of non-glycosylated human FS antigens has recently been described by others using MoAbs [3]. The alpha-spectrin [31] and actin previously identified in the human sperm tail principal piece [11] are more likely to be involved in the subcytoplasmic membrane cytoskeleton [4] than the FS structure.

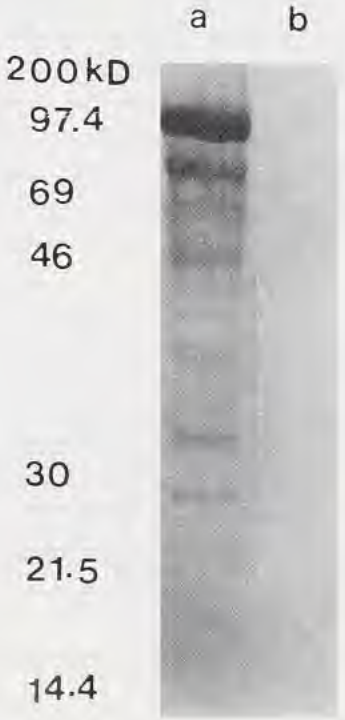

FIG. 2. — **a:** Western blotting and immunostaining of purified FS with MAFA xenoantisera; **b:** negative control. From [23], reproduced by permission of *Journal of Reproductive Immunology*.

During the immunogold EM investigations to localize the target antigens for GDA-J/F3, RT97, AJ-FS1, AJ-FS9 and MAFA, the gold labelling was seen to be restricted to the outer FS surface, i.e. the inner FS surface was not stained [14, 15, 17, 19, 23]. This antigenic polarity of the FS surfaces is determined by the locality of the antigenic epitopes which may incorporate bioactive groups e.g. phosphates or sugars [20, 16]. The presence of these groups together with the intimate association of the FS to the cytoplasmic membranes [24] may indicate some molecular interaction between these two structures during sperm motility.

Ontogeny and post-translational modifications of the FS antigens

The monoclonal and polyclonal anti-FS antibodies were useful for assessing the ontogeny of their target antigens during spermatogenesis. Thus, the restricted reaction of the GDA-J/F3, RT97, AJ-FS1, AJ-FS9 and MAFA with the mature sperm tails and lack of staining of the earlier germ cell stages indicated the late expression of the antigenic epitopes which followed the FS assembly [14, 15, 17, 19, 23]. These data are in contrast to those reported in mice [29] and rats [7, 10, 27]. In mice, for instance, the target antigen for K32 MoAb was first detected in the cytoplasmic matrix of stage 14 and 15 spermatids which then became localized to the FS at stage

16 [29]. This discrepancy could be due to the degree of maturation of the cells used in immunization; the epididymal and/or testicular germ cells preparations of rodents are likely to contain cells which are less mature than the ejaculated human sperm samples.

Following the human FS structural assembly, some of its proteins undergo post-translational modifications including phosphorylation [20], glycosylation [16] and disulphide bonding [2]. The phosphorylation and glycosylation were demonstrated by using RT97 and GDA-J/F3 MoAbs which recognized phosphorylated and glycosylated antigens respectively. These two processes take place in the testis prior to the release of spermatozoa to the epididymis and continue thereafter, whereas the disulphide linking of the FS protein occurs in the epididymis [2]. Although the biological importance of these post-translational modifications is yet to be established, their late occurrence following the FS assembly indicates their involvement in the FS molecular maturation to ensure a functional structure. Phosphorylation of the AJ-p97, for instance, is thought to contribute to the elasticity of the FS through the creation of electrostatic repulsion between the negatively-charged phosphate groups [20], whereas the FS rigidity could be due to the S-S bonding of the FS proteins [2].

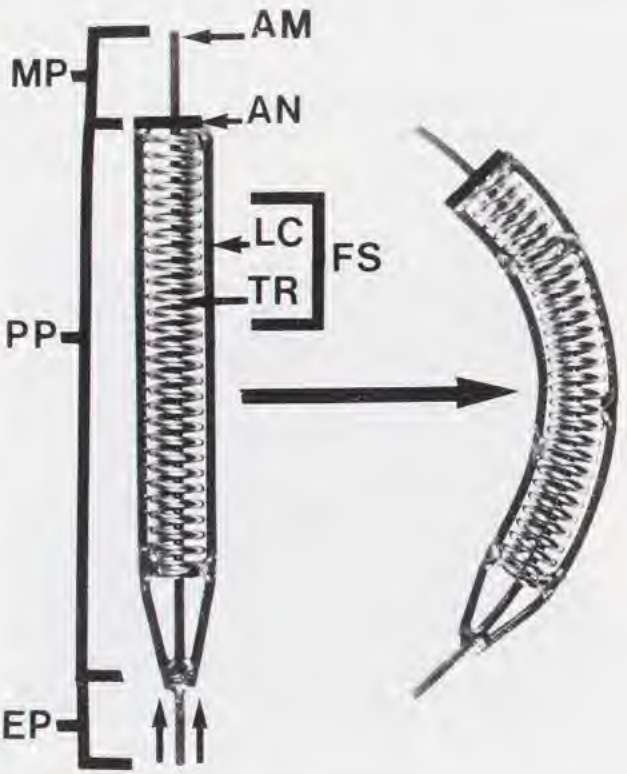

FIG. 3. — Illustration of the FS function. The attachment of the longitudinal columns (LC) to the axonemal microtubules (AM) ensures transmission of the microtubule linear sliding thrust to the FS which bends due to its spring-like features and produces a wave. AN, Annulus; EP, PP, and MP, end-, principal- and middle- piece of sperm tail; TR, Transverse ribs.

The biological function of the FS and its analogy to intermediate filaments

Because of their failure to react with anti-keratin, -vimentin and -neurofilaments antibodies, the human germ cells were previously reported to lack intermediate filaments [1, 12, 25]. However, despite the lack of the conventional antigenic determinants of intermediate filaments, the FS still shares several features with these cytoskeletal elements including: tissue specificity of its antigens [14-17], disulphide cross-linking [2], phosphorylation [20], and insolubility in various detergents and chemicals [22]. Furthermore, the FS develop as filamentous structures although their lumens are obliterated during development [8]. The diameters of the FS transverse rib and longitudinal column tubules are 5-6 nm [28] and 15-20 nm respectively [9], whereas that

of the somatic intermediate filaments is 10-15 nm [30]. All these features indicate that the FS could represent a modified form of intermediate filament. The AJ-p97 which shares a number of features with the neurofilaments including phosphorylation could, therefore, be the FS intermediate filament protein [14, 20]. However, amino acid and/or DNA sequencing is essential to establish its relationship to other intermediate filaments.

Although sperm motility is attributed to the axonemal microtubules, the presence of an additional structure is essential to convert the nonprogressive microtubular sliding into wavy movement. This structure is likely to be the FS due to its anatomical and structural properties. The FS is characterized by its spring-like morphology, rigidity and flexibility, and its location in a confined space; its anterior end starts at the annulus whereas its two longitudinal columns terminate posteriorly by attaching to microtubule doublets 3 and 8. This direct attachment to the microtubules ensures the transmission of the microtubular sliding thrust to the FS, and because of its presence in a confined space and spring-like features, the FS is likely to bend; consequently, the linear movements of the microtubules are converted into waves (Fig. 3). During bending, the proximation of the negatively-charged groups, e.g. the phosphates of AJ-p97, might ultimately lead to electrostatic repulsion and reversal of the original act [20]. In ciliated epithelium, the lack of the FS could be the reason for the inability of cilia to produce waves despite the presence of other axonemal structures.

Current investigations

The lambda gt11 human testicular cDNA libraries have recently been screened with AJ-FS1 MoAb, and two clones identified; their DNA sequence is currently being investigated (unpublished data). This is essential to assess any DNA sequence homology with other cytoskeletal proteins within the sperm tail and somatic cells. Future transfection of suitable host cells with the encoding genes could provide some clues to the synthesis and assembly of the FS proteins. Similar work of amino acid and/or DNA sequencing should be carried out in other species to evaluate their phylogenetic relationship.

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